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SURVEY AND SUMMARY

Compilation and analysis of σ^{54}-dependent promoter sequences

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ABSTRACT

Promoters recognized by the RNA-polymerase with the alternative sigma factor σ^{54} ($E\sigma^{54}$) are unique in having conserved positions around –24 and –12 nucleotides upstream from the transcriptional start site, instead of the typical –35 and –10 boxes. Here we compile 186 –24/–12 promoter sequences reported in the literature and generate an updated and extended consensus sequence. The use of the extended consensus increases the probability of identifying genuine –24/–12 promoters. The effect of several reported mutations at the –24/–12 elements on RNA-polymerase binding and promoter strength is discussed in the light of the updated consensus.

INTRODUCTION

The upstream regulatory region of all bacterial genes or operons contains the promoter, that is the DNA sequence which determines specific recognition by the RNA polymerase to initiate transcription. The eubacterial RNA polymerase is a heterotetrameric enzyme constituted by one β, one β' and two α subunits (1). This 'core' enzyme interacts with the initiation factor σ to form the transcriptionally active holoenzyme (Eσ). The addition of the σ factor confers upon the core the specificity for the recognition of promoter sequences.

All known σ factors belong to two different families: those evolutionarily related to the *Escherichia coli* housekeeping factor σ^{70}, and those related to the alternative factor σ^{54} (2). Each family of σ factors has different promoter sequence recognition, isomerization and regulation properties. $E\sigma^{70}$ does not form stable closed-promoter complexes, therefore transcription can be initiated spontaneously in the absence of activator proteins (3). In contrast, $E\sigma^{54}$ forms physically detectable closed-promoter complexes but it is unable to initiate transcription spontaneously. This polymerase is absolutely dependent on additional transcriptional factors, denominated enhancer binding proteins (EBPs), to initiate RNA synthesis (4). $E\sigma^{54}$ controls several ancillary processes including the degradation of xylene and toluene, transport of dicarboxylic acids, pilin synthesis, nitrogen fixation, hydrogen uptake (reviewed in 5,13), flagellar assembly (6), arginine catabolism (7), alginate production (8), rhamnolipid production (9), acetoin catabolism (10), mannose uptake (11) and proline iminopeptidase activity (12).

The basic promoters recognized by the $E\sigma^{70}$ family, although diverse in sequence, are normally configured around two hexamers centered between –10 and –35 nucleotides upstream from the transcriptional start site (14). In contrast, $E\sigma^{54}$ recognizes promoter sequences with conserved GG and GC elements located –24 and –12 nucleotides upstream from the transcriptional start site +1, that is one DNA helical turn closer than the recognition elements in –35/–10 promoters (15). The aim of the present work was the compilation and analysis of –24/–12 promoter sequences reported in the literature, either based on experimental analysis or identified by sequence similarity, and the discussion of possible regulatory implications. With the information obtained we refined the consensus sequence of σ^{54}-dependent promoters and carried out a comparative analysis of the effect of reported mutations affecting some of these promoters.

THE –24/–12 PROMOTER CONSENSUS SEQUENCE

We used three criteria to identify putative –24/–12 promoters: (i) mapped transcriptional start site with or without additional genetic evidence for the promoter; (ii) genetic evidence (mutation or heterologous gene expression); and (iii) putative promoters reported in the literature on the basis of sequence similarity to the –24/–12 elements. A collection of 186 sequences satisfying any of the three selection criteria is shown in Table 1. The nucleotide sequences were obtained from GenBank through the National Institute of Health server (http://www3.ncbi.nlm.nih.gov/entrez/). The sequence of each promoter including 10 nucleotides upstream and 10 nucleotides downstream from the highly conserved –24/–12 elements is shown in Table 1. For 85 of these promoters their transcriptional start site has been experimentally determined. A list of identified EBPs activating each promoter or the presence of putative EBP binding sites is included in Table 2 for the cases reported. The relative nucleotide frequency at each position and the consensus sequence for each subgroup and for the whole collection is shown in Figure 1.

The consensus sequences shown in Figure 1 are depicted following a previously reported definition (14), where any nucleotide occurring with a frequency of more than six

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Table 1. Nucleotide sequence of the 186 –24/–12 promoters used in this study

GENE	ORG.	-24/-12 PROMOTERS WITH MAPPED TSS		
			-24	-12
hoxF	*Al.eut*	ggtttcccttaggccctgcgaaatTGGCGCacatcCTGCGttccacctgcgcA		
hoxK		ggtttttccccaggtcttcggattcAGGCATagatGTTGTTTcaactatgtcG		
acoD		cgttttttcgttcgattcacgcccCGGCACagctcTTGCGatctgcctgccG		
acoX		gctgttttcactgcctgcgcgcacTGGCACagccaTTGCAaatacgcctggcA		
glnB	*Az.bra*	cagccggatcgcaaccgctccgatTGGCACgcaacGTGCTttacatcggA		
nifH		atgatttaaaaatttttcgcgaacTGGCACgggggATGCAgagaaggggT		
glnB	*Az.cau*	gtacgatctctgcaatggcacgaaTGGCATacatcGTGCTtacagttgtgcG		
nifA		gccgcttcagggaacaccgacaggAGGCTGatcccTCGCAgccgcttttgcG		
nifB	*Az.vin*	ttcaaaaacaattacttgaataatCGGCACgggtaTTGCTcggctcttgG		
anfH		tgtttggtggcgcggccggtaaacTGGCACatgcaTTGCTttatatacgA		
nifH	*Az.chr*	aggttatgtgtttgcctcgagtgcTGGCACagacgCTGCAttaggttcaG		
levD	*Bc.sub*	tgaaaacgcttaacacaactgtgtTGGCACgatccTTGCAttatatatggatG		
rocD		tagaaaacacacgaatttcttgatttGGCACagaacTTGCAtttatataaaggG		
rocA		TTTTTTCagcaaaagacaagaaaaTGGCATgattcTTGCAttttattcataT		
glnII	*Br.jap*	aaaacctcccgcaatgcggccttttGGCACgctaaATGCTtgtaaacggT		
fixB		actgacttcaatcgtctttgcaacTGGTACgacacTTGCTgttgtcctgC		
fixR		gaagcgcatttacgtacatcgcagTGGCGCaaatcCTGCTacgcgtgcgcG		
glnB		ccaataatcagggcatttgcaactTGGCATagaccCTGCTttcgaggaagC		
nifB		cattcgcgtcatcctcgccacGGcatgcaagttGCtaatcctcctgaA		
nifD		ctagttttagtgctcatgagacccTGGCATgccggTTGCAaagtcttggA		
nifH		agcttaaggtgccgggttagacctTGGCACggctgTTGCTgataagcgG		
nifS		tgagcatcatcagcgtctgcgcgcTGGCACgtgacTTGCTcttagctggG		
ndp		attgattgcactcgatttcaccttTGGCACgatacATGCGtaatcgtctaaA		
groESL3		agggcagatcgcttagcgcatttcTGGCCTgactcTTGCTacgggctgcttG		
fixA		gcgagcggtcccaagcggcgggggaTGGTACaagacTTGCTgttctcttccC		
hupS		cacttttgaatcgctccaggctgtTGGCCTgcttcTTGCTgtccttggcgtcA		
nifD	*Cw.rhi*	ctgtttttgcgccaaagacaggcccTGGCATgctggTTGCAaaagtcttgatcaA		
nifH		acgggcaagcgtTGGCACggcggTTGCTgataagcggC		
flgE	*Cm.col*	aaattcataaaaaatttcacaagtTGGAACagaacTTGCTtgtaaacT		
flaB		aaaatttcaatttgaatcaaaactTGGAACacttcTTGCTttaatcttttcG		
flaG	*Cl.cre*	aggtttaggtcgtgttttccgagtTGGCCCgaccgTTGCTgagggagG		
flbF		aaaatttggttaacaagatggcggCGGGCCgcaaaTCGCTccaagcgaaagC		
flbN		ccggcgccgccgctgtcggcccgcAGGCCCagagcTTGCAagcccgctcgcA		
flgF		ttgattttaatggcattttagcgcTGGCACgaaggTTGCTtctgggggttgG		
flbG		cgcgtcgccgggccgcgccggctTGGCGCcgtccTTGCGgaagacctT		
pillin		gctggtgggcagaatTGGCCCggcaaTTGCTtcaggtcctA		
flgH		ccggcgccgccgctgtcggcccgcAGGCCCagagcTTGCAagcccgctcgcA		
fdhF	*Es.col*	cagggaatgaccccacataaaatgTGGCATaaaagATGCAtactgtagtcA		
hycA		ctttaataacaataaattaaaagtTGGCACaaaaaATGCTtaaagctggcA		
hypA		attcaccgcagggatatcaacacTGGCACaattaTTGCTtgtagctggcA		
glnA		gccttttaggggcaatttaaaagtTGGCACagattTCGCTttatcttttT		
nac		tgtacatcaacaccaaaataaaacTGGCAAgcatcTTGCAatctggttgtA		
glnH		cgaaaaatcaaggagttgcaaaacTGGCACgatttCTTCAtatatgtgaatG		
pspA		ttataaatcaaaaagataaaaaatTGGCACgcaaaTTGTAttaacagttcA		
nac	*Kl.aer*	gcgacagccgaggaaatgcaaagcTGGCAAgcaaaTTGCAaaagccagtgcA		
glnA	*Kl.pne*	gccttttgggggcaatttaaaagtTGGCACagattTCGCTttatattttttT		
nifB		tcatgcggttgcgaaattaacctcTGGTACagcatTTGCAgcaggaaggT		
nifE		tctttaaaaatcaaggctccgctcTGGAGCgcgaaTTGCTgatgcaaaT		
nifF		gggccgcggtagtgcaaagcaaccTGGCACagcctTCGCAataccccTgC		
nifH		taagaatacataaacaggcacggcTGGTATgttccCTGCActtctctgctG		
nifJ		ctaactcttctgtcatccgcgagcTGGCACaggctGTGCTtgaggcaaC		
nifL		tgtttctgcacatcacgccgataaGGGCGCacggtTTGCAtggttatcaC		
nifM		tgatccccatcacccagccgtggcTGGCCGgaaatTTGCAatacagggaT		
nifU		ttgaaaaatattaatttattctcTGGTATcgcaaTTGCTagttcgttaT		
nifL	*Kl.oxy*	tgtttctgcttattacgccgataaGGGCGCacggtTTGCAtggttatcaC		
mbhA	*Mx.xan*	ggggcttggagtgcgcacctgggtTGGCATgcgtaGTGCTAatcccatccgcG		
4521		ctggaattcacttggccgcgcgtcGAGCACgcgtcTTGCTtttggctcacggcT		
pilA		gtcactgccgaattttgtcagcccTGGCATgcgtagTGCTaatcccatccgcggG		
pip	*Ns.gon*	ttgatggtttttatcgcgatttttCGGCATttgccTTTCGgggcggctttgtG		
nifD	*Pr.rhi*	ctgcttttttgtgctcgcgccgcgcTGGCATgctcgTTGCAgtcttgttcaA		
nifH		aagcttaataaggcgcggacagtgtTGGCATggcgaTTGCTgttgagT		
algC	*Ps.aer*	cggtcagtatcttcaggaactcggCGGGCAacgcaCTGCCaaaccccctG		
oprE		cgctcagtgtttcaacgaccggtcGGGCCCacgcgTTCCActtcccccG		
cpg2		tcggcggggcgaaggcaccgcagTGGCACtcgaaTTGCTataagaaccatgG		
rhlAB		acaagaacgaactctacgtaatgcCGGGATacccgTGGCAgcgatagctgttT		
algD		tatttcgcgagcgggacaaacggcCGGAACttcccTCGCAgagaaaaca-N10-G		
fleSR		ttgattttcaaatgaaaaaaatttAGGCACgggtaTTGCtatatctccgtcG		
xylA	*Ps.put*	aaataaggggatcggtataagcaaTGGCATggcggTTGCTagctatacgagA		
xylS		taaaaagaacgtcttcgttctgctTGGCGTtattTTGCTtggaaaagtG		
glnII	*Rh.leg*	ttttctttcgcgtgggcccaaaacTGGCACgctacGTGCTtttaaagcatC		
glnB	*b.vic*	ttttcgagaagatggatttcaaacTGGCACgatatCTGCAtcatatccggcG		
nifH		tgttcgtcagctcaatcggccgcttTGGCACgaatcTTGAGagctattgagaG		
glnII	*Rh.mel*	aagtggcgacgccccaaagcgagtTGGCACgttTgATGCTtaaggcaaatG		
fixA		attgatatttcgatattatcctgtTGGCACagcgcTTGCTtttttggacG		
nifH		ccgagtagttttatttcagacggcTGGCACgacttTTGCAcgatcagccctG		
nfe1		ttcgttccagccactcctgctgccCGGCACgacttTTGCAagatcacccgG		
nfe2		cattgcttcagtcactctgcggccTGGCATgacttTTGCAcgattatcccT		
p1		ccgagtagttttatttcagacggcTGGCACgacttTTGCAcgatcagcccT		
nifH	*Rh.BT*	gaaggaagcacgggcaagcgtTGGCACggcggTTGCTgataagcggC		
nifH	*Rb.cap*	gtcattatttgcgaaaattccggtTGGCACgatggCTGCTgtagaagctgt		
fdxD		GccggcaagcccgcccggcggcgcTGGCATggcccTTGCTgcgcggttttcG		
glnB	*Rs.rub*	ccacttgggtgatcattgaaccagTGGCACgaggcGTGATaagggatccccA		
nifJ		ttgcagaggtgggctcaggcgtccTGGAGAcgcgaCAGCGctccggG		
cheAY	*Rs.cen*	gcacggtgcgggtcgtccgcgaggGGGCAGgggtgTCGCTCcagggC		
glnA	*Sl.typ*	gccttttgggggcaatgtgaaagtTGGCACagattTCGCTttatattT		

GENE	ORG.	-24/-12 PROMOTERS WITHOUT MAPPED TSS		
			-24	-12
azu	*Al.den*	acttgtgcgtggcggcagcgacgcAGGCATgtgccTGGCGcgagtcgaag		
ureD1	*Al.eut*	gctgcgcggcagcgccgggatttgTGGGTCatacgCTGCTtacgcgaagc		
nifH	*A.fae*	gtgcttagattattttagaaacgcTGGCACaaaggCTGCTattgccctgt		
gltB	*Az.bra*	gggttccaggggtccatttcggccTGGCGGgggtgAAGGGgcgaagtcc		
orf2		atttaaaatcaaagacttaaaatttTGGCACgctgcTTGCTgatcctatga		
nifW		ccccgccgttaagcgcgatccggcTGGACGacctgCCGCTcgacggcatc		
nifB		ctgaaactccacacttcacccggtTGGCCCcgaacTTGCTttaacgacc		
fixA	*Az.cau*	cgcgttttcgggctgaaagacagtTGGTACgacacTTGCTcattcctccc		
nifO		ccgcggactggccatcgccagtacTGGCACgggcaTTGCTtggacctcat		
fixA	*Az.vin*	tataaaaatcctctgtttcgggtgTGGTACggctgTTGCAgtttcactgt		
nifE		ccttttaatcaggcgcttgccttcTGGTACaggcaTTGCAatgatccgtt		
nifF		taaatgtgttcgggggtttggggagTGGTCTgcttcTTGCTgttactcacc		
nifH		ggtttatttatgtgttgcgggtgcTGGCACagacgCTGCAttacctttgg		
nifH2		caatcatcgacttatcgatccttgTGGCACcgcccTTGCTcaactctcgt		
nifU		gaatgccaccaacagttgtggttgTGGCAAgtctTTGCTgttgaataa		
nifM		accatgtccacttcggcgacggtcTGGTATtgcagGTGCCcgagcagagc		
orf11		tgatttaaaggaaatattttccaaTGGCATcccaaTTGCAtatggccggt		
orf10		ggcgcaagcggggccgcaaggcgcAGGCAGgcgacATGCAcgagcggccc		
orf12		attagttaaacaacgaaatcctttCGGCACacttaTTGCAataggttgtc		
orf5		ctgtttttttctcaatttaacgcatTGGCAAagtctTTGCActccagatgc		
orf6		agataaaatttttaaacatatggcgTGGCATgccggTTGCAgcaatacctt		
orf8		cgtcgggccgtacccactccggggTGGCCGagtgaTCGCTggaaccacgg		
vnfENX		tcaggggacgcgggtcttgcgcgatTGGCGCgaccTTGCTccagctcctg		
anfA		gacatatatatcgataaggcgggcTGGCCCtaaaaTTGCTcttactcga		
vnfA		gacatatatatcgataaggcgggcTGGCCCtaaaaTTGCTcttactcgag		
vnfD	*Az.chr*	attccgcaacgccgcaagggcatcTGGCACgcatcCTGCCatttcctctt		
vnfH		caacatatgaaattcacatcttgaTGGCACcacccTTGCTccatcccctg		
nifH		caacatatgaaattcacatcttgaTGGCACcacccTTGCTccatccccctg		
nifM		accacgtgcatttcggcgacgggcTGGTGCtgcagGTGCCcgagcagagt		
nifU		gaatgccagcaacagctgtggttgTGGCAAgtctTTGCTtgctgattag		
flgL	*Cl.cre*	ttttcctcgattttggcgcggggcTGGCCCgccgcTTGCGatggttcgc		
flbG		aggtttaggtcgtgttttccgagtTGGCCCgaccgTTGCTgagggaggc		
flgI		agggcttggacacgctcataaaacTGGCCCgcttcTGGCAtgggcggcg		
celY	*Er.chr*	gcagcgtcagatggcgcatggcgaTGGCGGcggtgTTGCAgggggctctac		
hrpN		agtatccatcatgatgcctacatcGGGATCggcgtGGGCAtccgttgcag		
argT	*Es.col*	tgaatgttactgttgtcgtcaagaTGGCATaagacCTGCAtgaaagagcc		
fixA		aatcagtttgttatgttcttttgtGGGTAAaaaaaaTAGCAtctgactttc		
nifA	*Hr.ser*	cgagatcgattcatgcatctgcttGGGCATgaagtTTGCTtgaccagcaa		
nifB		attgaattgattgattatttttgtcTGGCACggtttTGGCTatgtcctcag		
nifH		tggcttggaatcgttttttgatggTGGCACggcatTTGCAaagagaggtg		
nifLA	*En.clo*	gttaacgcctgttaatgccgcagaAGGTGCactctTTGCAtggttaatgt		
nifL	*En.agg*	cctttgggcagataagtccgcgaaAGGCCTgcaaaTTGCAgggttattac		
nifB		aacaatttatatcatttttttcaggCGGTATaacctTTGCAgatattcaga		
nifH		aatgggtttacagctttggcatggTGGTACAaacaCTGCAatagcctgta		
nifE		gtttaaaatcagcaggtaatccgcTGGCCTgctccTTGCAaccttcaccc		
nifI		ttgaaataacgctgataatgtgacTGGCATcacaaTTGCTcagtagtcat		
nifJ		atatattaatacaacccttctgtcTGGCATgtcccATGCTtgttccttc		
nifZ		ataacgaccgcgcgcccctggctgcTGGCGCgaagaCTGCTcgcggagagc		
nifB	*Kl.oxy*	tcatgcggttgcgaaattgacctcTGGTACagcatTTGCAgcaggaagat		
nifF		gggccgcggtagtgcaaagaaaccTGGCACagcctTCGCAataccccTgt		
nifH	*Mb.the*	tgatctggtttctgttcatgtttaAGGATAtctgaGCGCAtaatttttcag		
glnA	*Mc.cap*	tgcgccattcgaaacgaaaacgaaTGGCACttaacTCGCGggtaaaatca		
glnA	*Pt.vul*	gtatatatccctttttatataagtTGGCATggtttTCGCAatagtatttt		
phhA		ggggctgcacggaccgtcccgacgCGGTATcgataAGGCAacgacaacaa		
azu		ggacgttgcgtcgcaggccgcgaagCGGCACatctgTGCTAaaacaggagt		
nirA		aatgtcgatctacgttcagggctgTGGAGCcgaggTTGCTccgcttcttg		
dmpK	*Ps.put*	accttgaagtctgttttcagacctTGGCACagcccTTGCTtgatgtcctg		
hrpL	*Ps.syr*	agtaacttaaataattatttttggcTGGCACggttaTCGCTatagggcttg		
avrD		cagagccacaatttttctaaaaacttTGGTGGctgtaTAGCTtcagcagtcg		
pelS		ccttggcaacaggatgtttgctgtGGGATAcgcagTTGCAataactgcac		
fixA	*Rh.leg*	atcttcttgcggcgcaaccttttatTGGCACgagtaGTGCTcagagtaagc		
orf240		atcctcaagcggcgctggggcatgCGGGCCatgcgGGGCAtgccggacg		
fixW		caccagcgtttgttgtccaaccgaTGGAACgagcgCCGCTcttcgaaa		
dctA		atcggttggttggcgactaaaacTGGCAGggcgaTTGCGaaggaggtgg		
fixZ		gtgccctcttcgaccgggcgccgaTGGCTCcctctATGCCcggcggccgc		
glnA		ttttcgagaagatggatttcaaacTGGCACgatatCTGCAtcatatccgg		
fixA	*b.pha*	atgtgatagagaacgccaaacggtTGGCACgaatgATGCAcgcaagcatg		
nifB	*b.tri*	ccggcaaggtgacgtttctcagacTGGCATcgctcTTGCTcgcggaactg		
nifB2	*b.vic*	cttaaagaaatcccgtccttgggaTGGCATggctttTTGCGttttgaaagg		
nifH1		ttccttggctcgatcggcccacaTGGCACgggttTTGAAgattgccatg		
nifHa	*Rh.pha*	ttccttggctcgatcggcccacaTGGCACgggttTTGAAgattgccatg		
nifHc		gtgttttatatgctgctgcccacaTGGCACgggttTTGAAgattgccatg		
D-ala	*Rh.mel*	ggggttgaccactgatcgctttgaAGGAAGaaaggCGACAgggcactgga		
dctA		acagcagtggctgccttgtggagcTGGTCAgcaacATGCGtgccagtttg		
dctB		tcgcataggccttccgcggcaaacTGGCACgcatgTTGCTgcaaagctcc		
fixD		attaagcgggcgagaaaatgactaAGGTGCtcccaTCGCAactcgttcag		
glnB		tgcgtcgtcattcctctacaaaagTGGCACgctacGTGCAtagtgtgagg		
mosB		attattttagtaactcccctcggccTGGCACgacttTTGCAcgatcagccc		
nifB		gccaatccattgacgaaaaggatttGGCATagctgTTGCTggttgaattg		
nifN		gtctttagctgaagtaaacaaaatTGGCACgagttTTGAGattcccggtc		
nifH	*Rh.sp*	gttctgcctatcaatcagctcaatTGGCACgacgcTTGAAaattgttcac		
nifH	*Rh.tri*	ttctctggctcaccctcctcgactTGGCACggcttTTGAAgccttccttg		
nifQ	*Rh.pNg*	accctcggctcagcagactggatTGGCACgaatTTGAAacgccccccgg		
fixA		attgatatttcgatattatcctgtTGGCACagcgcTTGCTtttttggacg		
fixB		attgatatttcgatattatcctgtTGGCACagcgcTTGCTtttttggac		
nifE		ctgcaaagaccgggtcgttctcaccTGGCACgttgcTTGCTtcatccctcc		
nifB	*Rb.cap*	gtgccaatggcccccgccgcgggccTGGCACggttcTTGCTgcatccctga		
orf6		cttgctttccccgcccgcatggtTGGCACgcttcTTGCTgcatccctga		
nifU		cttgcttgcgggggcccgttacggtTGGCACgcttcTTGCTggatcatttc		
nifF		ggcttcgaaaccgcgcgcaaagccTGGCACgaagCTTGCGaagcaactgc		
anfH		gggcatttcgccccgaagccgcctTGGCACggggcTGCTctataggggtt		
nifH	*Rs.rub*	caaaaaaatcgggcaaatcaggttTGGCACgctggGTGCATtaacggggt		
nifH	*Th.fer*	gtgttaaataggccatgatgaactTGGCACggcccTTGCAacagcgagga		
ntrB		caggggctgtgttcctgacggtcTGGTGCgctttTTGCTtactgagca		
argT	*Sl.typ*	cgaatcgttttgctgacgtgaaaaTGGCATaagacCTGCAtgaaaaagtc		
dhuA		gctttgccttgtcggcctgattaaTGGCACgatagTCGCAtcggatctgt		
prpB		taatttttctcgctgcctctttgccTGGCATagcctTTGCTttggtgaata		
glnA	*Vb.alg*	ttgaaattaatggaatatttttttTGGCACggtttTGGCTttagttaaaa		
flrB	*Vb.cho*	tgttgattttaaacatgaatggctTGGCATgactcTTGCTgtgccaagcca		
flaA		gagcgaagtgagttgagtaaaaagTGGCACggaagTTGCTaatacaaca		
rfaD		ggtctcgctcccaatagcgttctgTAGGATctcttTTGCAacgaagtct		

standard deviations from the expected random occurrence of each nucleotide (0.25) is denoted highly conserved (upper case), between three and six standard deviations is denoted weakly conserved (lower case), and below three standard deviations is not significant (N). As previously reported, the highest conservation was found around the –24 and the –12 elements (15). Around the –24 element, from position –31 to –20, there are eight highly conserved and three weakly conserved nucleotides with the sequence mrNrYTGGCACG (Fig. 1). Around the –12 element (positions –15 to –8), there are five highly conserved and one weakly conserved nucleotides with the sequence TTGCWNNw. Thus, the mrNrYTGGCACG-N4-TTGCWNNw sequence is the updated consensus for the –24/–12 promoters.

The subgroup with mapped transcriptional start site presented the extended consensus sequence but not the subgroup without mapped transcriptional start site (compare Fig. 1B and C). This discrepancy probably reflects the inclusion of incorrectly assigned –24/–12 promoter sequences in the compilation of the latter group. When the consensus sequence is displayed as the most frequent dinucleotide (Fig. 1D), it becomes apparent that at some positions one type of dinucleotide is strongly favored. For example, at position –14 T is by far the most abundant nucleotide (85%) but the presence of a pyrimidine is almost 100% conserved. Similarly, at positions –20 and –21 R and Y are >90% conserved.

A comparison of the consensus sequence derived by group and the relative occurrence of the most common nucleotide at each position is shown in Table 3. In the subgroup with mapped transcriptional start site, the G at the –24 position is 100% conserved; the G at the –25 position is 99% conserved, with the exception of the *Myxococcus xanthus* gene 4521 promoter (16,17); the G at the –13 position is 96% conserved with the exception of the *Pseudomonas aeruginosa oprE* (18), the *E.coli glnH* and the *Neisseria gonorrhoeae pip* (12,19) promoters; whereas the C at the –12 position is 96% conserved with the exceptions of the *Alcaligenes eutrophus hoxK* (20), the *E.coli pspA* (21), the *Rhizobium leguminosarum* biovar viciae *nifH* (22) and the *Rhodospirillum rubrum glnB* (23) promoters.

CONTACTS BETWEEN σ^{54} AND THE PROMOTER IN CLOSED COMPLEXES AND RELEVANCE OF EACH POSITION

Comparison of the deduced protein sequences of the σ^{54} factors showed that they are highly conserved (24): two well characterized motifs, a helix–turn–helix and a highly conserved sequence of 10 amino acids known as RpoN-box, are involved in the recognition of the –24 and –12 promoter elements, respectively (25–27). The promoter residues

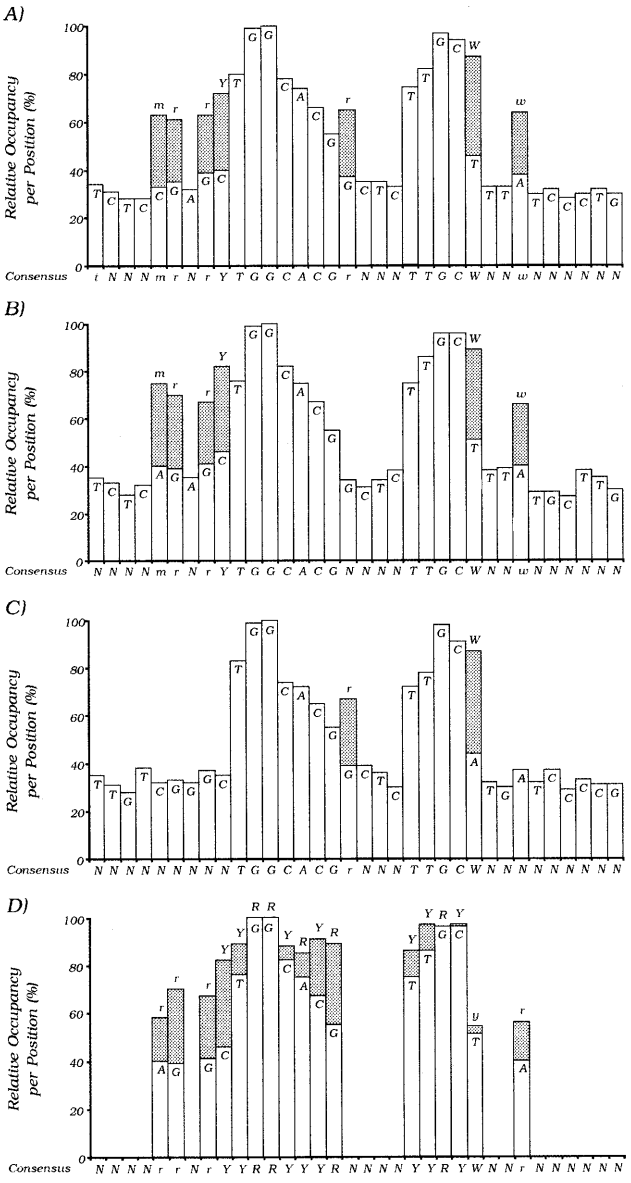

Figure 1. Relative frequency of each nucleotide per position and consensus sequences of the –24/–12 promoters. (**A**) Entire collection of the 186 –24/–12 promoters and putative promoters; (**B**) the 85 promoters with mapped transcriptional start sites; (**C**) the 101 putative promoters without mapped transcriptional start sites; (**D**) dinucleotide frequency composition of the 85 promoters with mapped transcriptional start sites. R, purines; Y, pyrimidines; W is A or T. Poisson statistics were used to express the standard deviation for each nucleotide (53).

(Table 1, opposite) The first column is the gene name and the second column the organism name abbreviated as follows: Al.den, *Alcaligenes denitrificans*; Al.eut, *Alcaligenes eutrophus*; A.fae, *Alcaligenes faecalis*; Az.bra, *Azospirillum brasilense*; Az.cau, *Azorhizobium caulinodans*; Az.vin, *Azotobacter vinelandii*; Az.chr, *Azotobacter chroococcum*; Bc.sub, *Bacillus subtilis*; Br.jap, *Bradyrhizobium japonicum*; Cw.rhi, Cowpea *Rhizobium*; Cm.col, *Campylobacter coli*; Cl.cre, *Caulobacter crescentus*; En.agg, *Enterobacter agglomerans*; En.clo, *Enterobacter cloacae*; Er.chr, *Erwinia chysanthemi*; Es.col, *Escherichia coli*; Hr.ser, *Herbaspirillum seropedicae*; Kl.aer, *Klebsiella aerogenes*; Kl.oxy, *Klebsiella oxytoca*; Kl.pne, *Klebsiella pneumoniae*; Mb.the, *Methanobacterium thermoautotrophicum*; Mc.cap, *Methylococcus capsulatus*; Mx.xan, *Myxococcus xanthus*; Ns.gon, *Neisseria gonorrhoeae*; Pr.rhi, Parasponia *Rhizobium*; Pt.vul, *Proteus vulgaris*; Ps.aer, *Pseudomonas aeruginosa*, Ps.put, *Pseudomonas putida*, Ps.syr, *Pseudomonas syringae*; Rh.leg, *Rhizobium leguminosarum*; b.pha, *Rhizobium leguminosarum* biovar *phaseoli*; b.tri, *Rhizobium leguminosarum* biovar *trifolii*; b.vic, *Rhizobium leguminosarum* biovar *viciae*; Rh.mel, *Sinorhizobium meliloti*; Rh.pha, *Rhizobium phaseoli*; Rh.sp, *Rhizobium* sp.; Rh.BT, *Rhizobium* BTAi1; Rh.tri, *Rhizobium trifolii*; Rh.pNG, *Rhizobium* plasmid NGR2; Rb.cap, *Rhodobacter capsulatus*; Rs.rub, *Rhodospirillum rubrum*; Rs.cen, *Rhodospirillum centenum*; Th.fer, *Thiobacillus ferrooxidans*; Sl.typ, *Salmonella typhimurium*; Vb.alg, *Vibrio alginolyticus*; Vb.cho, *Vibrio cholera*. TSS, transcriptional start site. Promoter sequences from 47 organisms are represented in our study. However, for only 33 of them has the *rpoN* gene been identified and sequenced.

Table 2. Regulatory features derived from the analysis of the 186 –24/–12 promoters used in this study

Organ.	Gene	TSSD	EBP	Bin.site	Distance	Reference
Al.den	*azu*	-	.	IHF	49	(56)
Al.eut	*hoxF*	14pb		IHF,HoxA	40,143	(58,59)
	hoxK	13pb				(20)
	ureD1	-		NtrC	163	AEY13732
	acoD	13pb				(10)
	acoX	14pb				(65)
Al.fae	*nifH*	-		NifA	100	(66)
Az.bra	*glnB*	11pb			96	(68)
	gltB			IHF,NifA	31,174	(70)
	ORF2	-		NifA	76	(72)
	nifW	-				(73)
	nifB	-		NifA	64,105	(75)
	nifH	11pb		NifA	70,79	(77)
Az.cau	*fixA*	-		NifA		(79)
	glnB	13pb				(80)
	nifO	-				(79)
	nifA	13pb				(81)
Az.vin	*fixA*	-				X65515
	nifB	11pb		NifA	95	(82)
	nifE	-		NifA	63	(84,85)
	nifF	-	NifA	NifA	113	(87)
	nifH	-		IHF,NifA	87	(85)
	nifH2	-				(89)
	nifM	-				(85)
	nifU	-				(85)
	anfH	11pb		IHF,AnfA	63,212	(91,92)
	ORF11	-		NifA	45	(85)
	ORF10	-		NifA	81	(85)
	ORF12	-		NifA	106	(85)
	ORF5	-		NifA	86	(85)
	ORF6	-		NifA	94	(85)
	ORF8	-		NifA	87	(85)
	vnfENX	-				(100)
	anfA					(85)
	vnfA	-				(85)
Az.chr	*nifH*	11pb		IHF,NifA	19,100	(104)
	vnfD	-				(106)
	vnfH	-				(107)
	nifH	-				(111)
	nifM	-				(113)
	nifU	-				(113)
Bc.sub	*levD*	14pb	LevR	IHF,LevR	41,80	(38,116)
	rocD	14pb	RocR	RocR	69,110	(7)
	rocA	14pb	RocR	IHF,RocR	18,88,132	(7,118)
Br.jap	*glnII*	11pb		NtrC	78	(120)
	fixB	11pb				(121)
	fixR	12pb	NifA	RegR,NifA	43,60,81	(122,123)
	glnB	12pb		NtrC		(124)
	nifB	13pb				(120)
	nifD	11pb		NifA	69	(125,126)
	nifH	10pb		IHF,NifA	71,100	(128,129)
	nifS	11pb		NifA	52	(130)
	ndp	13pb		IHF,NifA	46	(132)
	groESL3	13pb				(134)
	fixA	12pb				(135)
	hupS	14pb				(137)
Cw.rhi	*nifD*	15pb		NifA	71	(139)
	nifH	11pb				(139)
Cm.col	*flgE*	9pb				(140)
	flaB	13pb				(141)
Cl.cre	*flaG*	9pb				(41)
	flgL		FlbD		82	(143,144)
	flbF	13pb				(147)
	flbN	13pb				(148)
	flbG		FlbD	IHF	75	(41,43,150)
	flgF	12pb	FlbD	IHF	21,51	(150,152,153)
	flaN	10pb	FlbD	IHF,ftr	54	(41,43)
	flgI	-	FlbD	IHF	16,37,71	(150,153,154)
	pillin	11pb				(156)
	flgH	13pb	FlbD	ftr	88	(150,153)
Er.chr	*celY*	-				(159)
	hrpN					(161)
Es.col	*argT*	-				(162)
	fdhF	12pb		FhlA	84	(163,164)
	fixA	-				(166)
	hycA	12pb		IHF,FhlA	6,56	(168,169)
	hypA	12pb		FhlA		(169,171)
	glnA	12pb		NtrC	76,108	(3,173,174)
	nac	12pb		NtrC,NAC	42,118	(176)
	glnH	13pb		IHF,NtrC	74,86	(19,178)
	pspA	12pb	PspF	IHF,PspF	2,63,84	(21,179)
Hr.ser	*nifA*	-		NifA	96	(181)
	nifH	-		NifA	82,112	(182)
	nifB	-		NifA	55,97	(181)
En.clo	*nifLA*					(184)
En.agg	*nifL*	-		NtrC	163	(185)
	nifB	-		NifA	93	(185)
	nifH	-		NifA	94	(189)
	nifE	-				X99694
	nifI	-				X99694
	nifJ	-		NifA	107	(189)
Kl.aer	*nac*	13pb		NtrC,NAC	42,119	(176,194)
Kl.pne	*glnA*	12pb		NtrC	74	(163)
Kl.pne	*nifB*	11pb		IHF,NifA	91	(129,142)
	nifE	11pb		IHF,NifA	3,33	(130,197)
	nifF	11pb		NifA		(57)
	nifH	12pb		IHF,NifA	9,96	(60,61)
	nifJ	10pb		IHF,NifA	14,107,153	(61)
	nifL	11pb		NtrC	137	(62,63)
	nifM	11pb				(64)
	nifZ	-				(64)
	nifU	11pb		IHF,NifA	2,76,85	(67)
Kl.oxy	*nifL*	11pb				(69)
	nifB	-		NifA	91	(69,71)
	nifF	-				(69)
Mb.the	*nifH*	-				(74)
Mc.cap	*glnA*			NtrC	41	(76)
Mx.xan	*mbhA*	14pb				(17,78)
	4521	14pb				(16,17)
	pilA	16pb				(17)
Ns.gon	*pip*	13pb				(12)
Pr.rhi	*nifD*	12pb		NifA	69	(48)
	nifH	8pb				(48)
Pt.vul	*glnA*	-				(83)
Ps.aer	*algC*	11pb	AlgR	AlgR	61,146	(8,86)
	oprE	10pb			187	(18)
	PhhA		PhhR	PhhR	51	(88)
	cpG2	14pb				(90,93)
	azu	-				(56)
	nirA	-				M97294
	rhlAB	14pb	RhlR	RhlR	13,151	(9,93)
	algD	21pb	AlgR	IHF	33,391,468	(51,55)
	fleSR	13pb	FleQ	IHF,NifA	5,42	(6)
Ps.put	*dmpK*	-		IHF	28	(94)
	xylA	13pb		IHF,XylR	33,100,131	(95-97)
	xylS	11pb		IHF,XylR	6,113,143	(97-99)
Ps.syr	*hrpL*	-				L36536
	avrD	-				(101)
	pelS	-				(102)
Rh.leg	*fixA*	-				(103)
	orf240					(105)
	fixW	-				(103)
	dctA	-	DctD	DctD	75,108	(108,110)
	fixZ	-				(112)
	glnA	-				(114)
	glnII	12pb				(115)
b.pha	*fixA*	-		NifA	67	(117)
b.tri	*nifB*	-				X16311
b.vic	*glnB*	13pb				(119)
	nifB2	-			70	L11084
	nifH1	-		NifA	91	L11084
	nifH	13pb		NifA	85	(22)
Rh.pha	*nifHa*	-		NifA	90	(127)
	nifHc	-				(127)
Rh.mel	*D-ala*	-				(131)
	glnII	12pb		NtrC	73	(133)
	dctB	-				(109)
	dctA	-				(108,136)
	fixA	13pb		NifA	77	(138)
	fixD	-				(60)
	glnB	-				U50385
	mosB	-				U23753
	nifH	13pb				(60)
	nifB	-		NifA	41,61	(142)
	nfe1	12pb		NifA	34	(145,146)
	nfe2	12pb		NifA	88	(145,146)
	nifN	-		NifA	89	(149)
	P1	12pb		NifA	81	(151)
Rh.sp	*nifH*	-		NifA	67	(155)
Rh.BT	*nifH*	11pb				(157)
Rh.tri	*nifH*	-		NifA	88	(158)
Rh.pNG	*nifQ*	-				(160)
	fixA	-		NifA	74	(160)
	fixB			NifA	74	(160)
Rb.cap	*nifB*	-				(165)
	nifE					(167)
	nifH	13pb		IHF,NifA	49	(129,170)
	ORF6	-			39	(172)
	nifU	-		NifA	41	(175)
	nifF			NifA		(177)
	fdxD	13pb				(170)
	anfH					(180)
Rs.rub	*glnB*	13pb		NtrC	79	(23)
	nifH	-		IHF,NifA	90,121	(129,183)
	nifJ	8pb				(50)
Rs.cen	*cheAY*	8pb				(49)
Th.fer	*nifH*	-		IHF,NifA	74,119	(186-188)
	ntrB			NtrC	115	(186)
Sl.typ	*argT*	-				(190)
	dhuA	-		NtrC	103	(190,191)
	glnA	9pb		NtrC	74	(192)
	prpB	-				(193)
Vb.alg	*glnA*	-				(146)
Vb.cho	*flrB*	-				(195)
	flaA	-				(196)
	rfaD	-				(198)

Table 3. Comparison of –24/–12 promoter consensus sequences

COMPARISON OF σ^{54}-DEPENDENT PROMOTER SEQUENCES

	-25	-24														-13	-12											
A)	N	N	N	N	N	N	N	N	N	T	G	G	C	A	C	G	r	N	N	N	T	T	G	C	W	N	N	N	N	N	N	N
										83	99	100	74	72	65	55	67				73	79	99	92	87							
B)	N	N	N	N	m	r	N	r	Y	T	G	G	C	A	C	G	N	N	N	N	T	T	G	C	W	N	N	w	N	N	N	N	N
					75	70		67	82	76	99	100	82	75	67	55					75	86	96	96	89			66					
C)	t	N	N	N	m	r	N	r	Y	T	G	G	C	A	C	G	r	N	N	N	T	T	G	C	W	N	N	w	N	N	N	N	y
	34				63	61		63	72	80	99	100	78	74	66	55	64				74	82	97	94	87			64					62
D)	N	N	N	N	m	r	N	r	Y	T	G	G	C	A	C	G	N	N	N	N	T	T	G	C	W	N	N	w	N	N	N	N	N

Numbers under the sequences represent the relative occurrence of the nucleotide at that position (percent).
A, consensus sequence from the 186 compiled promoters.
B, consensus sequence from 85 promoters with mapped transcriptional start site.
C, consensus sequence from 101 putative promoters without mapped transcriptional start site.
D, consensus sequence obtained from the comparison of groups A, B and C.

contacted by $E^{54}\sigma$ have been extensively described (28–31): σ^{54} contacts actually extend from positions –31 to –5, although only for the GG and GC dinucleotides at positions –24 and –12 have specific protein–DNA interactions been detected (30). Further addition of the core polymerase induces a distortion downstream from the –12 element but the reactivity towards methylating reagents did not change, indicating that the core subunits do not contact the promoter directly but through σ^{54} (29,30,32). It has been proposed that the –24 element functions as an attachment determinant for $E\sigma^{54}$ whereas the –12 element is involved in the fine modulation of an already established closed-complex towards its isomerization (33).

Mutations reported elsewhere helped to identify positions critical for the promoter function (16,27,34–38). Table 4 shows several reported mutations in –24/–12 promoters and a quantitative analysis of their effect. All the changes in the highly conserved positions –25/–24 and –13/–12 are down-mutations, reducing drastically the binding of $E\sigma^{54}$ (31,33) and the expression, except for the *M.xanthus* 4521 gene promoter which has an A at –25 (Table 4, lane E) (39). Qualitatively, similar changes reported in the *Azorhizobium caulinodans nifA* (40), the *Caulobacter crescentus flbG* and *flaN* (41–43) and the *Klebsiella pneumoniae nifH* (44) promoters, also presented a down-phenotype. Mutations in the *K.pneumoniae nifH* promoter at the –26 and –16 positions decreased the expression by 25% and at the –15 position by 75% (35,37), whereas simultaneous mutations at the –17, –16 and –15 positions decreased the expression by 55% (Table 4, lane C) (35). The substitution of the latter residues for three Ts enhanced the σ^{54} and $E\sigma^{54}$ binding affinities both *in vivo* and *in vitro* (31,40,45). Mutations around the –24 region that increased the similarity to the consensus; for example, a change of G for A at the –22 position in the *K.pneumoniae nifL* promoter, enhanced the expression level more than 2-fold (34).

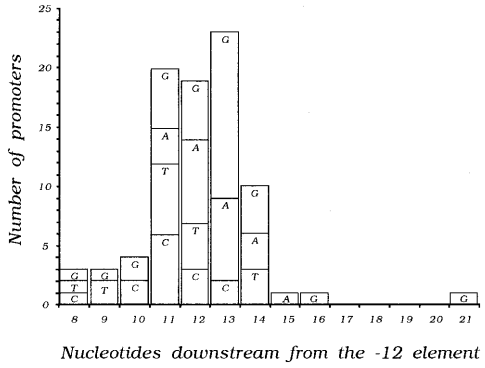

Figure 2. Distribution of transcriptional start sites for the 85 –24/–12 promoters. The reference position is the conserved C of the –12 element.

TRANSCRIPTIONAL START SITES IN –24/–12 PROMOTERS

σ^{70}-Dependent promoters generally initiate transcription at a purine, adenine being more frequently utilized than guanine (14). The selection of this nucleotide is influenced by the sequence around –35 and by the composition of the –2 to –5 positions (46,47). In our collection of 85 σ^{54}-dependent promoters with a mapped transcriptional start site a significant proportion of them (64%) also initiate transcription at a purine (Fig. 2). In σ^{70}-dependent promoters the spacing between the first nucleotide of the –10 element and the transcriptional start site is usually 6 or 7 nt, although functional examples between 4 and 10 nt have been reported (14).

The first group of –24/–12 promoters being characterized initiate transcription precisely 12 nt downstream from the

(Table 2, opposite) Organism names are as in Table 1. TSSD, distance from the transcriptional start site to the C of the conserved –12 element; EBP, experimentally identified activator of the EBP family; Bin.site, possible EBP binding site identified by sequence similarity; Distance, the distance from the second G of the –24 element to the EBP binding site.

Table 4. Effect of some mutations in –24/–12 promoters

		-25	-24											-13	-12						
A)	T	G	G	C	A	C	G	N	N	N	N	T	T	G	C	T/A	N	N	A	N	N	N
B) *Kp-nifL*	G	G A (3)	G A (4)	C T (118)	G A (261)	C T (116)	A	C T (112)	G A (83)	G A (93)	T	T	T	G A (3)	C T (13)	A	T	G	G	T	T	A
C) *Kp-nifH*	T C (75)	G	G	T	A	T	G	T	T A (102)	C T T (109)	C T T (78)	C T T (24)	T (45) C (8)	G A (1)	C T (7)	A	C	T	T	C	T	C
D) *Bs-levD*	T	G	G C (1) A (1) T (2)	C	A G (17)	C	G	A	T	C	C	T	T A (2)	G A (1) C (1)	C	A	T	T	A	T C (18)	A	T
E) *Mx-4521*	G A (99)	A T (76)	G T (4)	C	A T (9)	C A (9)	G T (13)	C	G T (128) A (122)	T C (76)	C	T	T	G T (2) C (5)	C	T C (29)	T	T A (147)	G T (135)	G A (116) T (102)	C T (81)	T C (62)
F) *Kp-glnA*	T	G A (12) C (12) T (11)	G A (10) C (14) T (9)	C	A	C	A	G	A	T	T	T	C	G A (6) C (14) T (26)	C A (12) G (12) T (14)	T	T	T	A	T	A	T

Relative values (%) are shown in parentheses.
A, consensus sequence from the –24/–12 promoters with mapped transcriptional start sites derived from this analysis.
B, mutant forms of the *K.pneumoniae nifL* promoter (34,37).
C, mutant forms of the *K.pneumoniae nifH* promoter (35–37).
D, mutant forms of the *B.subtilis levD* promoter (38).
E, mutant forms of the *M.xanthus* 4521 gene promoter (16).
F, mutant forms of the *K.pneumoniae glnA* promoter (27).

conserved GC element; this coincidence led to the operational designation of these promoters as –24/–12 (15). Our updated compilation of 85 promoters with mapped transcriptional start sites from 28 different species provides evidence that the initial nucleotide selection is more flexible than initially considered. In Figure 2 we present these data as a Gaussian distribution similar to that obtained from the analysis of σ^{70}-dependent promoters from *Bacillus subtilis* and *E.coli* (48,49), with the significant difference that instead of the preferential use of a single position, the use of nucleotides 11, 12 and 13 downstream from the GC for initiation of transcription is roughly equally frequent (24, 20 and 27% respectively).

A few examples of transcripts starting 3 nt before and after positions 11–13 have been also described and were included in the analysis. The shortest distance reported is 8 nt for the *Parasponia rhizobium nifH* (50), the *Rhodospirillum centenum cheAY* (51) and the *R.rubrum nifJ* (52) promoters. At present, there are no reported examples of transcriptional starts between 17 and 20 nt. The longest distance reported is 21 nt for the *P.aeruginosa algD* promoter (53,54). Based on these results it is not possible to predict accurately a transcriptional start site for –24/–12 promoters based solely on the promoter sequence.

SPACING BETWEEN THE –24 AND –12 CONSERVED ELEMENTS

The optimal spacing between the –35 and –10 elements in σ^{70}-dependent promoters is 17 +/– 1 nt, but functional promoters with spacing between 15 and 20 non-conserved nucleotides have been reported (14,55). In contrast, deletions of one or more nucleotides in the stretch between the –24/–12 elements abolished promoter function (16,36,41–43). Thus, a stringent requirement for these motifs to be positioned on the same face of the DNA helix seems to be a necessary condition for the binding of Eσ^{54}. Although there are no published examples of nucleotide insertions between the –24/–12 elements, it is reasonable to predict that any insertion might severely disrupt promoter function.

SUMMARY AND CONCLUSIONS

Here we present an updated compilation of σ^{54}-dependent promoters and the derivation of an extended consensus sequence. The new consensus extends from positions –8 to –31 relative to the transcriptional start site (Fig. 1 and Table 3). Interestingly, the observed contacts of σ^{54} with the promoter DNA span exactly this length (30). Although the informational content of the extended consensus sequence is still low to accurately predict –24/–12 promoters, profile searches using this sequence increased 4-fold the probability of identifying bona fide promoters in the bacterial subgroup of the EMBL/GenBank database, compared to the previous consensus (data not shown).

The consensus sequence derived from our collection of 186 –24/–12 promoter elements and putative promoter elements from 47 different bacterial species shows a remarkable conservation both in sequence and in structure in contrast to the flexible consensus derived from σ^{70}-dependent promoters. This conservation symbolizes the strict requirements for promoter recognition and function required for a highly controlled regulation.

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